Extracorporeal Gas Exchange and Spontaneous Breathing for the Treatment of Acute Respiratory Distress Syndrome: An Alternative to Mechanical Ventilation?*

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Objectives: Venovenous extracorporeal gas exchange is increasingly used in awake, spontaneously breathing patients as a bridge to lung transplantation. Limited data are available on a similar use of extracorporeal gas exchange in patients with acute respiratory distress syndrome. The aim of this study was to investigate the use of extracorporeal gas exchange in awake, spontaneously breathing sheep with healthy lungs and with acute respiratory distress syndrome and describe the interactions between the native lung (healthy and diseased) and the artificial lung (extracorporeal gas exchange) in this setting.

Design: Laboratory investigation.

Setting: Animal ICU of a governmental laboratory.

Subjects: Eleven awake, spontaneously breathing sheep on extracorporeal gas exchange.

Interventions: Sheep were studied before (healthy lungs) and after the induction of acute respiratory distress syndrome via IV injection of oleic acid. Six gas flow settings (1–10 L/min), resulting in different amounts of extracorporeal CO_2 removal (20–100% of total CO_2 production), were tested in each animal before and after the injury.

Measurements and Main Results: Respiratory variables and gas exchange were measured for every gas flow setting. Both healthy and injured sheep reduced minute ventilation according to the amount of extracorporeal CO_2 removal, up to complete apnea. However, compared with healthy sheep, sheep with acute respiratory distress syndrome presented significantly increased esophageal pressure variations (25 ± 9 vs 6 ± 3 cm H_2O; $p < 0.001$), which could be reduced only with very high amounts of CO_2 removal (> 80% of total CO_2 production).

Conclusions: Spontaneous ventilation of both healthy sheep and sheep with acute respiratory distress syndrome can be controlled via extracorporeal gas exchange. If this holds true in humans, extracorporeal gas exchange could be used in awake, spontaneously breathing patients with acute respiratory distress syndrome to support gas exchange. A deeper understanding of the pathophysiology of spontaneous breathing during acute respiratory distress syndrome is however warranted in order to be able to propose extracorporeal gas exchange as a safe and valuable alternative to

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mechanical ventilation for the treatment of patients with acute respiratory distress syndrome. (*Crit Care Med* 2014; 42:e211–e220)

Venovenous extracorporeal gas exchange (ECGE), also called "extracorporeal membrane oxygenation," is increasingly used as an adjunct to mechanical ventilation in patients with acute respiratory distress syndrome (ARDS) (1–4). Furthermore, ECGE has been successfully used in awake, nonintubated, spontaneously breathing patients as a bridge to lung transplantation (5–8) and starts to be used for the treatment of exacerbation of chronic obstructive pulmonary disease (9, 10).

The rationale for supporting gas exchange with ECGE alone, that is, without mechanical ventilation, in patients with ARDS is strong. Indeed, the avoidance of mechanical ventilation would have several advantages as it could potentially reduce the prevalence of ventilator-associated pneumonia and ventilator-induced lung injury. Furthermore, as the performance of active physical therapy was shown to be feasible while on ECGE (7, 11, 12), the reduction in depth of sedation allowed by the avoidance of mechanical ventilation could favor its performance. Nevertheless, limited data are available on the use of ECGE as an alternative to mechanical ventilation for ARDS patients (13), that is, for patients characterized both by acute impairment of CO_2 removal and severe hypoxemia.

If we consider the use of ECGE in awake, spontaneously breathing patients with ARDS, a new factor needs to be taken into account. Indeed, while the control of breathing has been thoroughly studied in physiological conditions and the key role of CO_2 has been identified (14, 15), less is known about the respiratory drive of ARDS patients. It is conceivable that neural pathways that are silent under physiological conditions—for example, bronchopulmonary C-fibers and other lung receptors (16, 17)—could be activated by lung edema, congestion, and inflammation and could influence the respiratory activity of patients with ARDS (18, 19).

The aim of the present work was to develop a model of awake, spontaneously breathing sheep on venovenous ECGE in order to study the interactions between the artificial and the native lung. We hypothesized that, in sheep with ARDS, gas exchange could be supported with ECGE alone and that spontaneous ventilation could be controlled via ECGE similarly to healthy sheep.

MATERIALS AND METHODS

This study was approved by the U.S. Army Institute of Surgical Research Animal Care and Use Committee and was conducted in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

Animal Preparation

Under general anesthesia, 11 mixed-breed female sheep (45 ± 6 kg) were tracheostomized. Catheters were placed in the right carotid artery and in the pulmonary artery (via left jugular vein). A balloon catheter (Ackrad Labs, Cooper Surgical, CT) was introduced transnasally in the esophagus (20) and respiratory system and lung pressure-volume (PV) curves were obtained, as previously described (21), starting from functional residual capacity. Chest CT (Toshiba Aquilion 64-slice Medical System, Tustin, CA) was performed (60 mAs, 120 kVp, pitch factor 0.85) at airway pressures of 0 and 30 cm H_2O for lung quantitative analysis (Maluna 3.17, Göttingen, Germany) (22, 23). A 23F bicaval dual-lumen catheter (Avalon Elite, Maquet, Rastatt, Germany) was placed via right jugular vein as previously described (24) and connected to the Cardiohelp (Cardiohelp, Maquet, Rastatt, Germany). Blood flow (BF) through the membrane lung (ML) was set at 2 L/min and kept constant throughout the study. Activated clotting time was kept greater than 160 with heparin infusion. Midazolam (0.05–0.20 mg/kg/hr) and buprenorphine (0.01 mg/kg every 4–6 hr) were administered for sedation and analgesia. For each sheep, the level of sedation was kept constant throughout the study.

Study: "Healthy Lungs"

Sheep were placed prone, awakened, weaned from mechanical ventilation and kept, via tracheostomy, on continuous positive airway pressure (CPAP) of 8 cm H_2O with F_{IO_2} of 0.5 (Evita XL, Dräger Medical, Germany). Control measurements were performed once the animals were stable (on average 180 min after instrumentation). Thereafter, six different sweep gas flows (GFs) (range, 1–10 L/min) and therefore different amounts of CO_2 removal by the ML ($V_{M}CO_2$) were randomly tested in each sheep (**Table E1**, Supplemental Digital Content 1, http://links.lww.com/CCM/A818); F_{IO_2} of GF was set at 0.5. Each setting was maintained for 30–40 minutes, at the end of which measurements were performed (*Data Collection* section). Every two steps, GF was zeroed to measure control conditions.

ARDS Induction and Study: "ARDS"

Once all measurements had been performed, F_{IO_2} was increased to 1.0 and ARDS was induced via IV oleic acid (OA) injection (0.1–0.15 mL/kg) (25, 26) with target Pao_2 less than 200 mm Hg. All GF settings were repeated in the same order of the "healthy study" and the same measurements were performed. Less time (15–20 min) was spent at 0 L/min of GF to avoid discomfort for the animals.

Data Collection

Respiratory variables, esophageal pressure variations (ΔP_{es}), arterial and mixed-venous blood gases, hemodynamics, V_{CO_2} (CO$_2$SMO, Novametrix, Wallingford, CT) and oxygen uptake (V_{O_2}) of the native lung ($V_{L}CO_2$, $V_{L}O_2$), and $V_{M}CO_2$ (VMax Encore, Viasys, Yorba Linda, CA) were recorded for every GF setting; V_{O_2} of the ML ($V_{M}O_2$) was measured at predefined GF settings (Table E1, Supplemental Digital Content 1, http://links.lww.com/CCM/A818). Pulmonary shunt fraction and physiologic dead space were calculated with standard equations. Blood chemistry and serum cytokines (interleukin
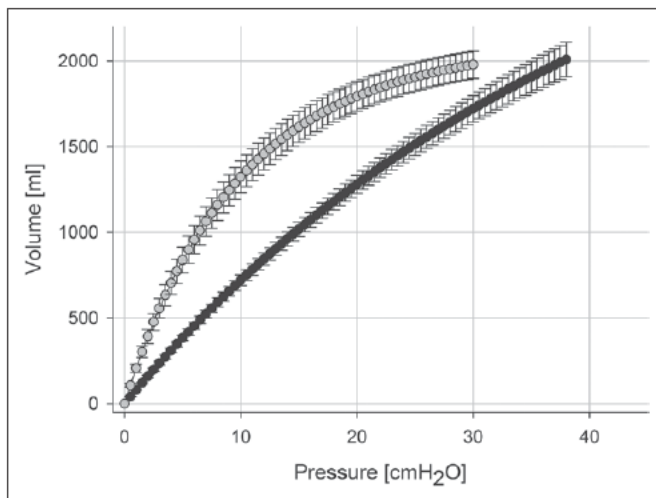
Figure 1. Average pressure-volume (PV) curve of the lung (*gray dots*) and respiratory system (*black dots*) of healthy sheep (*n* = 10). The curve was computed as previously described (21). Pressure represents transpulmonary pressure in the case of lung PV curve and airway pressure in the case of respiratory system PV curve. For clarity, data are expressed as mean ± SE.

[IL]-1β, IL-8, IL-6; tumor necrosis factor-α and IL-10, Mybiosource, San Diego, CA) were evaluated at baseline and end of study. CT was repeated under general anesthesia at the end of study (see **Fig. E1** for study design, Supplemental Digital Content 1, http://links.lww.com/CCM/A818).

Statistical Analysis

Data are expressed as mean ± SD unless otherwise stated. Variables recorded before (healthy lungs) and after the induction of ARDS were compared via paired *t* test or signed rank sum test, as appropriate. Categories of extracorporeal CO_2 removal were compared with one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. Tukey or Dunn test were used for post hoc multiple comparisons. Two-way ANOVA was used to assess interactions between groups (healthy sheep vs ARDS) and categories of extracorporeal CO_2 removal. A rank transformation was used for nonnormally distributed variables that did not pass the equal variance test. Slopes and intercepts of linear regressions were compared with the test for equality of slopes and intercepts, respectively. Statistical significance was defined as *p* value less than 0.05. Analysis was performed with SAS v.9.2 (SAS, Cary, NC) and SigmaPlot v.12.0 (Systat Software, San Jose, CA).

For additional details on the methods, see the **online supplemental data** (Supplemental Digital Content 1, http://links.lww.com/CCM/A818).

RESULTS

All sheep were placed uneventfully on ECGE and survived the injury. No ECGE-related complications were observed. During OA injection, a transient period (45–90 s) of apnea coupled

TABLE 1. Quantitative Chest CT Scan Results of Healthy Sheep and Sheep With Acute Respiratory Distress Syndrome

Quantitative CT results	Airway Pressure (cm H$_2$O)	Healthy	Acute Respiratory Distress Syndrome	*p*
Lung volume (mL)	0	2,435±680	2,017±454	0.006
	30	3,838±730	2,382±550	<0.001
Air volume (mL)	0	1,717±586	292±211	<0.001
	30	3,145±617	595±388	<0.001
Mean CT number (HU)	0	−696±47	−135±83	<0.001
	30	−819±11	−233±121	<0.001
Hyperinflated tissue (%)	0	0±0	0±0	0.24
	30	5±3	0±0	<0.001
Normally aerated tissue (%)	0	83±5	5±4	<0.001
	30	85±5	10±9	<0.001
Poorly aerated tissue (%)	0	16±5	22±12	0.19
	30	9±2	26±5	<0.001
Nonaerated tissue (%)	0	2±1	73±15	<0.001
	30	2±1	64±11	<0.001
Lung weight (g)		705±120	1,756±303	<0.001

Airway pressure = airway pressure at which the scan was performed (either 0 or 30 cm H$_2$O), *p* = *p* value of the comparison between values of healthy sheep and sheep with acute respiratory distress syndrome, lung volume = total lung volume, air volume = total volume of air of the lung, mean CT number = mean CT number of the whole lung expressed in Hounsfield Units, hyperinflated tissue = mass of hyperinflated tissue (density between −901 and −1,000 HU), normally aerated tissue = mass of normally aerated tissue (density between −900 and −501 HU), poorly aerated tissue = mass of poorly aerated tissue (density between −500 and −101 HU), nonaerated tissue = mass of nonaerated tissue (density between −100 and +200 HU), lung weight = weight of the lungs expressed in grams.

All compartments are expressed as percentage of total lung weight.

with a marked bradycardia followed by 10–15 minutes of rapid shallow breathing was observed in all sheep.

Description of the Study Population Without ECGE
The average lung and respiratory system PV curves of healthy sheep are shown in **Figure 1**.

Table 1 summarizes quantitative CT results before and after the induction of ARDS. Respiratory variables and gas exchange of healthy spontaneously breathing sheep and sheep with ARDS are reported in **Table 2**. All data were recorded at 2 L/min of BF (33% ± 9% of cardiac output) in the absence of sweep GF.

A significant increase in pulmonary pressure (16 ± 3 vs 21 ± 6 mm Hg; $p = 0.003$) was observed after the induction of ARDS. Furthermore, a reduction in cardiac output (6.3 ± 1.1 vs 4.6 ± 1.5 L/min; $p = 0.006$) and central venous (2 ± 5 vs -2 ± 5 mm Hg; $p = 0.03$) and pulmonary occlusion pressure (7 ± 3 vs 4 ± 4 mm Hg; $p = 0.03$) was recorded. These variations were likely due to intravascular hypovolemia induced by plasma leakage caused by OA (25) which explains also the increase in hemoglobin (9.9 ± 1.1 vs 12.0 ± 1.7 g/dL; $p < 0.001$) and hematocrit ($29.1\% \pm 4.3\%$ vs $37.1\% \pm 4.2\%$; $p < 0.001$). A significant reduction both in WBCs (4.1 ± 1.3 vs 1.0 ± 1.1 10^3/mL) and in plasma cytokines IL-1β, IL-8, and IL-10 (**Table 3**) was recorded.

Interactions Between Native and Artificial Lung
Figure 2A shows the response in terms of reduction in minute ventilation to different amounts of $V_M CO_2$ (expressed as percentage of total $V CO_2$ [$V_{TOT}CO_2$]) of healthy sheep. A good correlation ($r^2 = 0.74$; $p < 0.001$) was found between $V_M CO_2$ and reduction in minute ventilation. The equation of the overall regression ($y = -3.6 + 0.94x$) closely resembled the identity line.

Figure 2B shows the same graph for injured sheep. Also sheep with ARDS reduced their spontaneous breathing activity according to the amount of CO_2 removed extracorporeally ($y = -24.0 + 1.1x$; $r^2 = 0.59$; $p < 0.001$), up to complete apnea when $V_M CO_2$ approached total metabolic CO_2 production.

When comparing linear regressions of healthy and injured animals, no significant difference was observed for slopes ($p = 0.19$) while a significant difference was observed for the intercepts of the equations ($p < 0.001$).

Analysis by Categories of Extracorporeal CO_2 Removal
Experimental points were divided in three categories of $V_M CO_2$ (1–60%, 61–80%, and 81–100%, expressed as percentage of $V_{TOT}CO_2$), in order to analyze, for each group, the variations in respiratory variables and gas exchange in response to different

TABLE 2. Respiratory Variables and Blood Gases of Spontaneously Breathing Sheep Without Extracorporeal Gas Exchange

Variables	Healthy	Acute Respiratory Distress Syndrome	p
Respiratory rate (breaths/min)	24±5	62±27	< 0.001
Tidal volume (mL)	395±121	265±69	0.002
ΔP_{es} (cm H_2O)	6.1±2.8	24.7±8.7	< 0.001
Minute ventilation (L/min)	9.8±3.6	17.0±6.9	0.008
Alveolar minute ventilation (L/min)	5.7±2.7	2.7±1.5	0.003
Dead space fraction	0.44±0.10	0.81±0.10	< 0.001
Pulmonary shunt fraction	0.01±0.01	0.25±0.10	< 0.001
$V_L CO_2$ (mL/min)	230±106	145±49	0.002
$V_L O_2$ (mL/min)	185±63	189±83	0.41
Arterial pH	7.41±0.04	7.30±0.08	0.002
$Paco_2$ (mm Hg)	40.5±3.9	47.0±10.0	0.02
Pao_2 (mm Hg)	227±27	94±57	< 0.001
Pao_2:Fio_2 ratio	454±54	94±57	< 0.001
Sao_2 (%)	100±0	90±7	< 0.001
Svo_2 (%)	73±7	56±13	0.002
Base excess (mEq/L)	1.0±3.9	−3.0±6.1	0.008
Lactate (mmol/L)	2.1±1.5	3.4±2.6	0.14

$p = p$ value of the paired t test, ΔP_{es} = swing of esophageal pressure (surrogate of pleural pressure variation), dead space fraction = physiologic dead space fraction, shunt fraction = fraction of venous admixture, $V_L CO_2$ = carbon dioxide removed through the native lung expressed in mL/min, $V_L O_2$ = oxygen consumption expressed in mL/min.

Fio_2 was set at 0.5 for healthy sheep, while it was set at 1 after acute respiratory distress syndrome induction. Positive end-expiratory pressure was kept constant throughout the study at 8 cm H_2O.

TABLE 3. Plasma Cytokines in Healthy Sheep and in Sheep With Acute Respiratory Distress Syndrome

Cytokines (ng/mL)	Healthy	Acute Respiratory Distress Syndrome	p
IL-1β	97±46	66±53	0.02
Tumor necrosis factor-α	38±9	39±11	0.20
IL-6	263±274	211±217	0.12
IL-10	382±229	139±182	<0.001
IL-8	216±235	67±92	0.008

IL = interleukin.

Measurement of plasma cytokines was performed on a subset of animals ($n = 8$) for technical problems related to plasma storage of the first 3 experiments. Comparison was performed with the paired t test. For additional details on the methodology used for cytokine analysis, see the online supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A818).

amounts of V_MCO_2. Furthermore, data obtained at 0 L/min of GF, that is, V_MCO_2 of 0 mL/min (0% of $V_{TOT}CO_2$), were added to the analysis (when applicable) as a separate group. Results for healthy and injured sheep are reported in **Tables 4** and **5**, respectively.

By partitioning minute ventilation into its two components, tidal volume and respiratory rate, a slight difference between healthy and injured animals was found. Indeed, both respiratory rate variation (**Fig. 3**) and tidal volume variation (**Fig. 4**) differed significantly between the two groups.

For additional results, see the online supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A818).

DISCUSSION

We developed a model of awake, spontaneously breathing sheep on venovenous ECGE that allowed us to study the ventilatory response to different amounts of extracorporeal CO_2 removal both in healthy sheep and in sheep with OA-induced ARDS.

Previous studies, performed in similar experimental settings in healthy, spontaneously breathing lambs and sheep (14, 15), demonstrated the key role of extracorporeal CO_2 removal in the control of breathing. So far, however, no experimental data had been reported on the use of ECGE in spontaneously breathing animals with ARDS and on the interactions between the artificial lung and the native, diseased lung.

The response to different amounts of extracorporeal CO_2 removal was, in some respects, similar in healthy and diseased animals. Indeed, in both conditions sheep reduced minute ventilation progressively and accordingly to the amount of removed CO_2, up to complete apnea (Fig. 2). Furthermore, the response of healthy sheep was very similar to previous reports
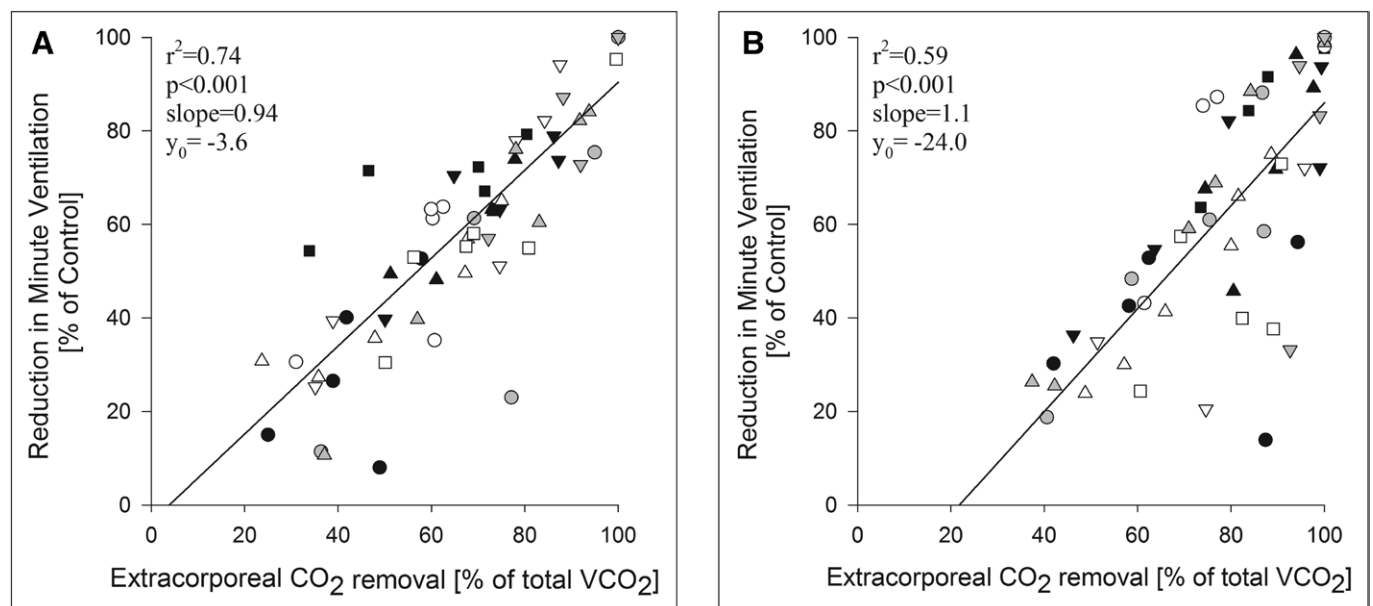
Figure 2. Correlation between extracorporeal CO_2 removal and reduction in minute ventilation in healthy sheep (**A**) and in the same sheep with acute respiratory distress syndrome (**B**). Extracorporeal CO_2 removal is expressed as percentage of total CO_2 production ($V_{TOT}CO_2 = V_MCO_2 + V_LCO_2$), while minute ventilation is expressed as percentage reduction compared to control values, that is, measured minute ventilation in the absence of extracorporeal CO_2 removal. Every symbol (combination of shape and color) represents, in both panels, experimental points recorded from the same animal. The regression lines refer to the overall population.

TABLE 4. Analysis by Categories of Extracorporeal CO_2 Removal in Healthy Sheep

Healthy Sheep (n = 11)	Extracorporeal CO_2 Removal (% of Total Vco_2)				
	0%	1–60%	61–80%	81–100%	
	(n = 11)	(n = 21)	(n = 22)	(n = 17)	p
$V_M co_2$ % (%)	NA	43±11[a,b]	70±6[a]	91±7	<0.001
Sweep gas flow (L/min)	NA	3.5±3.1[a,b]	7.1±3.7	7.3±3.7	0.001
$V_M co_2$ (mL/min)	NA	123±61[a,b]	180±49	175±35	<0.001
$V_L co_2$ (mL/min)	230±106[a,b,c]	158±69[a,b]	79±32[a]	18±17	<0.001
$V_{TOT} co_2$ (mL/min)	NA	281±115[a]	258±76[a]	193±42	0.008
$V_L o_2$ (mL/min)	185±64[a,b]	174±102[a]	124±50	96±54	<0.001
$V_{TOT} o_2$ (mL/min)	NA	227±111[a]	172±55	145±64	0.008
MV reduction (% of control)	NA	36±17[a,b]	60±13[a]	84±14	<0.001
MV_{ALV} reduction (% of control)	NA	39±21[a,b]	65±12[a]	90±8	<0.001
Tidal volume (mL)	395±121[a,b]	317±96[a,b]	231±112[a]	84±65	<0.001
ΔP_{es} (cm H_2O)	6.1±2.8[a,b]	5.6±2.5[a,b]	3.2±1.0	2.6±2.0	<0.001
Respiratory rate (breaths/min)	24±5[a]	23±5[a]	19±6	11±10	<0.001
Pulmonary shunt fraction	0.01±0.01[a,b]	0.01±0.00[a,b]	0.03±0.03	0.12±0.17	<0.001
Dead space fraction	0.44±0.12[a]	0.46±0.11[a]	0.52±0.11[a]	0.76±0.19	<0.001
Pao_2 (mm Hg)	227±27[a]	229±39[a]	214±29	168±64	0.001
$Paco_2$ (mm Hg)	40.5±3.9	38.1±4.3	37.3±4.8	36.4±4.7	0.13
Arterial pH	7.41±0.04	7.45±0.06	7.43±0.05	7.46±0.05	0.07

p = p value of the one-way analysis of variance, NA = not available, $V_M co_2$ % = amount of extracorporeal CO_2 removal expressed as percentage of total Vco_2, $V_M co_2$ = absolute value of extracorporeal CO_2 removal expressed in mL/min, $V_L co_2$ = Vco_2 of the sheep, $V_{TOT} co_2$ = total Vco_2, $V_L o_2$ = oxygen uptake of the sheep, $V_{TOT} o_2$ = total oxygen uptake (sheep + extracorporeal gas exchange, $V_M o_2$ did not change with gas flow and was considered constant for each sheep), MV reduction = reduction in minute ventilation expressed as % of control values, MV_{ALV} reduction = reduction in alveolar minute ventilation expressed as percentage of control values, ΔP_{es} = esophageal pressure swings (available in 10 animals).

[a] $p < 0.05$ vs. 81–100%.
[b] $p < 0.05$ vs. 61–80%.
[c] $p < 0.05$ vs. 1–60%.

Experimental measurements were divided into four categories of extracorporeal CO_2 removal: 0%, 1–60%, 61–80%, and 81–100%, expressed as percentage of total metabolic CO_2 production. The average value for each sheep of data obtained at 0 L/min of gas flow, that is, $V_M co_2$ of 0 mL/min (0% of $V_{TOT} co_2$), was used for analysis.

Data are expressed as mean ± SD.

(14, 15), with the overall regression equation being close to the identity line (Fig. 2A).

On the other hand, sheep with ARDS had a similar slope, but a significantly lower intercept (Fig. 2B). As the intercept of this graph represents the reduction in minute ventilation expressed as percentage of control (ventilation without ECGE), a negative value means that sheep with ARDS, on average, would have needed to breathe 20–25% more than their control ventilation to eliminate all metabolically produced CO_2. Furthermore, this means that 20–25% of total Vco_2 could be removed extracorporeally without a significant effect on minute ventilation. Above those values, pco_2 and pH were normalized and sheep responded to increasing CO_2 removal by reducing ventilation, up to apnea. However, in injured animals, the response to CO_2 removal was more heterogeneous, with some animals presenting significant spontaneous ventilation despite high amounts of CO_2 unloading. This observation, which might be explained by the influence on spontaneous breathing of factors not directly linked to gas exchange (agitation, metabolic status, and lung receptor activity), might be of potential clinical relevance as it suggests that the response of individual patients could differ despite similar circumstances.

An increase in pulmonary shunt fraction was recorded in all animals for high amounts of extracorporeal CO_2 removal. This fact, which was likely caused by pulmonary derecruitment/ atelectasis (14), might have been accentuated by high Fio_2 in sheep with ARDS. Lung derecruitment might not be tolerated by the sickest patients; however, it is conceivable that the application of higher levels of CPAP (27) or the use of biphasic positive airway pressure (28) could reduce/prevent its occurrence. Of note, injured sheep presented only moderate pulmonary shunt (25% ± 10%) despite the marked increase in pulmonary edema and the increase in poorly and nonaerated lung tissue (Table 1). This fact might be explained by some typical features

TABLE 5. Analysis by Categories of Extracorporeal Co_2 Removal in Sheep With Acute Respiratory Distress Syndrome

Acute Respiratory Distress Syndrome (n = 11)	Extracorporeal Co_2 Removal (% of Total Vco_2)				
	0%	1–60%	61–80%	81–100%	
	(n = 11)	(n = 12)	(n = 18)	(n = 31)	p
$V_M co_2$ % (% of total Vco_2)	NA	48±8[a,b]	71±7[a]	93±7	< 0.001
Sweep gas flow (L/min)	NA	2.1±1.0[a]	5.0±2.6[a]	8.2±3.2	< 0.001
$V_M co_2$ (mL/min)	NA	107±32[a,b]	176±47[a]	212±37	< 0.001
$V_L co_2$ (mL/min)	145±49[a,b]	116±35[a]	71±24	16±17	< 0.001
$V_{TOT} co_2$ (mL/min)	NA	223±58	247±61	229±43	0.40
$V_L o_2$ (mL/min)	185±80[a]	156±77[a]	94±40	75±58	< 0.001
$V_{TOT} o_2$ (mL/min)	NA	233±81[a]	169±37	151±56	0.004
MV reduction (% of control)	NA	32±9[a,b]	58±19[a]	79±24	< 0.001
MV_{ALV} reduction (% of control)	NA	39±20[a]	60±21[a]	81±25	< 0.001
Tidal volume (mL)	265±69[a]	248±48[a]	214±73[a]	113±89	< 0.001
ΔP_{es} (cm H_2O)	24.7±8.7	26.7±6.1	22.8±7.7	13.8±11.1	0.01
Respiratory rate (breaths/min)	62±27[a]	54±26[a]	38±24	23±25	< 0.001
Pulmonary shunt fraction	0.25±0.10[a]	0.23±0.12[a]	0.30±0.18	0.48±0.29	0.005
Dead space fraction	0.81±0.10	0.78±0.10	0.78±0.15[a]	0.90±0.12	0.008
Pao_2 (mm Hg)	94±57	119±98	118±80	89±58	0.45
$Paco_2$ (mm Hg)	47.0±10.0[a]	39.1±7.4	40.6±9.3	35.8±8.0	0.004
Arterial pH	7.30±0.08[a,b]	7.40±0.06	7.36±0.09	7.41±0.08	< 0.001

p = p value of the one-way analysis of variance, NA = not available, $V_M co_2$ % = amount of extracorporeal Co_2 removal expressed as percentage of total Vco_2, $V_M co_2$ = absolute value of extracorporeal Co_2 removal expressed in mL/min, $V_L co_2$ = Vco_2 of the sheep, $V_{TOT} co_2$ = total Vco_2, $V_L o_2$ = oxygen uptake of the sheep, $V_{TOT} o_2$ = total oxygen uptake (sheep + extracorporeal gas exchange, $V_M o_2$ did not change with gas flow and was considered constant for each sheep), MV reduction = reduction in minute ventilation expressed as % of control values, MV_{ALV} reduction = reduction in alveolar minute ventilation expressed as percentage of control values, ΔP_{es} = esophageal pressure swings (available in 10 animals).

[a] $p < 0.05$ vs. 81–100%.

[b] $p < 0.05$ vs. 61–80%.

Experimental measurements were divided into four categories of extracorporeal Co_2 removal: 0%, 1–60%, 61–80%, and 81–100%, expressed as percentage of total metabolic Co_2 production. The average value for each sheep of data obtained at 0 L/min of gas flow, that is, $V_M co_2$ of 0 mL/min (0% of $V_{TOT} co_2$), was used for analysis.

Data are expressed as mean ± SD.

of spontaneous breathing: preserved diaphragmatic activity and favorable ventilation/perfusion ratio (29).

High amounts of extracorporeal Co_2 removal were also associated to a significant increase in physiologic dead space, which was likely caused primarily by a relative increase in anatomic dead space fraction due to the reduction in tidal volume. Another possible factor contributing to the observed increase in physiologic dead space was the recorded increment in pulmonary shunt (30).

Another interesting finding was the analysis of the two components of Vo_2. On the one hand, oxygen delivery through the ML, that is, $V_M o_2$, was constant throughout the study phases (no correlation with $V_M co_2$) but increased significantly after ARDS induction (51 ± 18 vs 79 ± 17 mL/min; $p < 0.001$) due to increased hemoglobin concentration and reduced hemoglobin saturation of blood entering the ML (72% ± 8% vs 62% ± 10%; $p < 0.001$). On the other hand, a progressive and significant reduction in $V_L o_2$ was observed with increasing $V_M co_2$, resulting in a significant reduction in total oxygen consumption. The reduced oxygen consumption might be explained by a reduced respiratory muscle activity, that is, a lower cost of breathing (31). As a reduction in oxygen consumption also implies a lower Co_2 production, this effect could therefore potentially increase the relative contribution of ECGE. In our opinion, this "metabolic" effect of ECGE deserves attention, and its potential role in optimizing extracorporeal support needs to be defined, especially in patients supported with ECGE for longer periods.

When analyzing the relative contribution of tidal volume and respiratory rate to the reduction in minute ventilation due to Co_2 unloading (Figs. 3 and 4), we found slightly different responses in healthy and injured sheep. In healthy sheep, the reduction in minute ventilation was caused first by a reduction in tidal volume and only for higher values of $V_M co_2$ also by
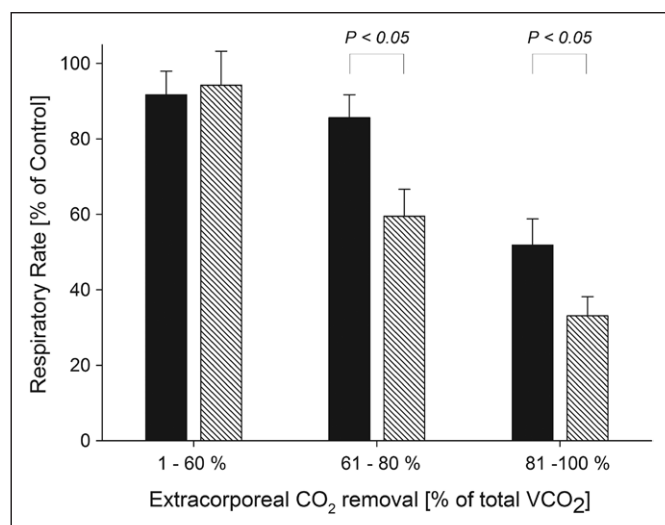
Figure 3. Variations in respiratory rate (expressed as % of control measurements) caused by different amounts of extracorporeal CO_2 removal expressed as percentage of total CO_2 production. Experimental points were grouped in three categories of extracorporeal CO_2 removal: 1–60%, 61–80%, and 81–100%. *Black bars* represent healthy sheep, while *hatched bars* represent sheep with acute respiratory distress syndrome. A two-way analysis of variance was performed ($p = 0.01$). Data are expressed as mean ± SE.

Figure 4. Variations in tidal volume (expressed as % of control measurements) caused by different amounts of extracorporeal CO_2 removal expressed as percentage of total CO_2 production. Experimental points were grouped in three categories of extracorporeal CO_2 removal: 1–60%, 61–80%, and 81–100%. *Black bars* represent healthy sheep, while *hatched bars* represent sheep with acute respiratory distress syndrome. A two-way analysis of variance was performed ($p = 0.001$). Data are expressed as mean ± SE.
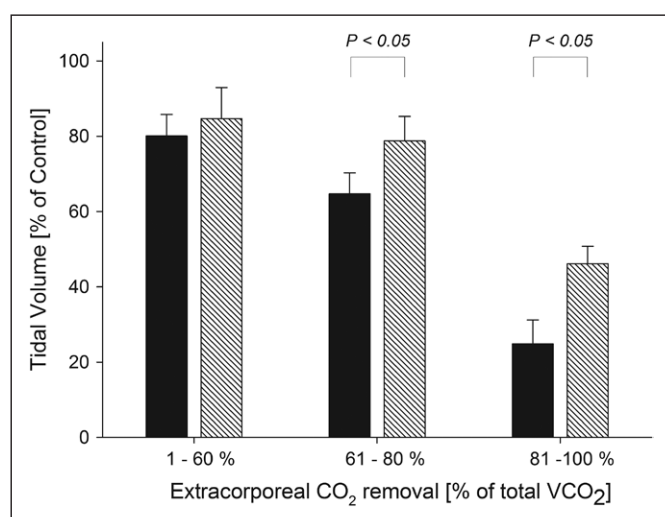
reduction in respiratory rate. On the contrary, in sheep with ARDS, the reduction in minute ventilation was caused first by a reduction in respiratory rate and only for higher values of $V_M CO_2$ also by a reduction in tidal volume.

Recorded tidal volumes without ECGE (GF = 0 L/min) were between 8 and 9 mL/kg in healthy sheep and between 5 and 6 mL/kg in sheep with ARDS. In fact, spontaneous tidal volumes recorded in sheep with ARDS could be considered "protective" for mechanical ventilation (32).

However, in injured animals at 0 L/min of GF, ΔP_{es} (a surrogate for transpulmonary pressures) was 24.7 ± 8.7 cm H_2O, which corresponds to a *dynamic* variation in transpulmonary pressure very close to the one recorded in *static* conditions in healthy animals when inflating the lung from functional residual capacity to total lung capacity (Fig. 1). If we assume that the portion of the lung still viable for ventilation, that is, the "baby lung" (33), has similar anatomical and physiological characteristics of healthy alveoli, we might hypothesize that those lung units were subject to a deformation that approached their own total capacity and that they could therefore be potentially subjected to cellular stress failure (34). Indeed, several studies that investigated spontaneous hyperventilation, be it experimental (35, 36), exercise-related (37, 38), or clinical (39), discussed the potential role of spontaneous alveolar stretching in the development of pulmonary edema that in fact did not differ from ventilator-induced lung injury. We must therefore be very cautious in assuming that the risk of ventilator-induced lung injury could be eliminated through the use of ECGE and the avoidance of mechanical ventilation, as spontaneous ventilation per se could also be potentially injurious ("*ventilation*-induced lung injury"). Furthermore, it is very important to underline the fact that sheep with ARDS reduced esophageal pressure variations and tidal volume only when very high amounts of CO_2 (> 80% of total $V CO_2$) were removed extracorporeally.

It might be of interest to speculate on the mechanisms that caused these high pleural pressure swings and this response pattern to extracorporeal CO_2 unloading. On the one hand, due to edema accumulation and lower lung compliance, higher pleural swings were necessary to be able to ventilate the injured lung. On the other, it is conceivable that other factors, for example, lung receptor activity, were involved. Indeed, while the control of breathing has been extensively studied in physiological conditions (40, 41), less is known about pathological situations in which neural pathways that are usually silent in physiological conditions may be active (18, 19).

In the present study, we have not evaluated the activity of pulmonary receptors or vagal afferents; however, we clearly observed, a few seconds after the injection of OA, the clinical manifestations of the pulmonary chemoreflex, that is, apnea and bradycardia followed by rapid shallow breathing. These phenomena have been attributed to the simultaneous activation of bronchopulmonary C-fibers (J-receptors) (42, 43). It is therefore likely that, because of both direct chemical activation and the ensuing activation caused by lung congestion, collapse, and microembolization (44), the activity of unmyelinated pulmonary fibers and other lung receptors was increased throughout the second study phase (ARDS) (18).

The role of cytokines in the pathogenesis of OA-induced ARDS is still debated (45). Indeed, some studies reported an increase in cytokines (46, 47), while others did not show any significant variation (48). Interestingly, we observed a reduction in several plasma cytokines (Table 4). These results are difficult to explain and their interpretation is of pure speculative

nature: on the one hand, it is possible that OA induced cellular lysis (reduction in WBCs) with ensuing reduction in circulating inflammatory mediators; on the other hand, a "cytokine catching" effect of the ML might be hypothesized.

Some important limitations of the present study need to be mentioned. First, the intrinsic limitations of the OA model need to be kept in mind (45). Indeed, the fact that inflammation is not a clear feature of the OA model limits the clinical translatability of our results to human ARDS.

Furthermore, we need to point out the relative short duration of the observations once ARDS was induced. In fact, we studied only the acute phase of ARDS. We therefore do not know if other factors could have a role in determining the respiratory pattern and response to extracorporeal CO_2 removal in later stages of the disease.

In conclusion, the rationale to use ECGE for the treatment of ARDS in spontaneously breathing patients as an alternative to mechanical ventilation is strong as it would allow to avoid several mechanical ventilation–associated side effects. Indeed, ECGE could be used both as first-line treatment in nonintubated patients with ARDS and to accelerate the weaning process in already intubated patients with respiratory failure. The risks associated with ECGE, especially bleeding and membrane failure, however, need to be kept in mind. Finally, in this scenario, a somewhat new player, namely spontaneous breathing, would enter in the arena of the ICUs.

This study sheds light on some aspects of the use of venovenous ECGE in spontaneously breathing patients with ARDS, but a deeper understanding of the pathophysiology of spontaneous breathing during ARDS and of the potential harm caused by high pleural pressure variations is warranted in order to be able to propose ECGE as a safe and valuable alternative to mechanical ventilation for the treatment of patients with ARDS.

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